Combinatorial-complete immunotherapy protocols: three proposed trial designs testing the seven-mode framework in immunotherapy-refractory cancers

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2026

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Abstract

The seven-mode framework for cancer immunotherapy (Monteiro 2026, Paper 3 in this series) predicts that combinatorial protocols engaging all seven failure modes of immune-mediated tumor control simultaneously will produce qualitatively different clinical outcomes from contemporary single-mode or narrow combination interventions. The framework's structural arguments are established. Its translational test

requires specific trial designs in tumor populations where multiple failure modes are operative and where component interventions exist but have not been combined. This paper proposes three such trials: (1) a combinatorial-complete protocol in BRCA1/2-mutant triple-negative breast cancer building on KEYNOTE-522; (2) an extended autogene cevumeran protocol in pancreatic ductal adenocarcinoma adding Mode 3, 5, and 6 engagement; (3) a multi-mode protocol in microsatellite-stable colorectal cancer building on the FOLFOX/bevacizumab standard. For each trial we specify population, intervention components mapped to the seven modes, comparator arm, endpoints, statistical design, biomarker strategy, anticipated toxicity, and feasibility. Each protocol uses FDA-approved or late-stage investigational components combined in ways that have not been systematically tested. The framework predicts these combinations will convert immunotherapy-refractory populations into immunotherapy-responsive ones. The trials are biologically rational, operationally feasible, and have not been run. They should be.

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1. Introduction

1.1 The translational gap the framework identifies

The seven-mode framework developed in Paper 3 of this series organizes cancer immunotherapy around seven structural failure modes of immune-mediated tumor control: insufficient antigen presentation, lack of T cell priming and DC engagement, physical exclusion of T cells, T cell exhaustion, active immune suppression by the tumor microenvironment, insufficient persistence of immune response, and tumor heterogeneity with immune escape. The framework's central prediction is that combinatorial coverage across all seven modes produces qualitatively different outcomes from interventions engaging subsets of modes.

The empirical pattern across 15 years of contemporary immunotherapy clinical development is consistent with this prediction. Tumors where intrinsic biology adequately addresses Modes 1, 2, 3, 5, 6, 7 respond dramatically to Mode 4 interventions (checkpoint blockade) — melanoma, MSI-high colorectal cancer, high-PD-L1 NSCLC. Tumors where multiple modes are operative as failure mechanisms remain immunotherapy-refractory despite intensive Mode 4 optimization — pancreatic ductal adenocarcinoma, microsatellite-stable colorectal cancer, much of triple-negative breast cancer, glioblastoma, castration-resistant prostate cancer.

The framework predicts that combinatorial-complete protocols — engaging 6 or 7 of the seven modes simultaneously — should convert these refractory populations into immunotherapy-responsive ones. The component interventions for such protocols exist. Every individual mode has FDA-approved or late-stage investigational agents that engage it. The combinatorial protocols testing the framework's prediction do not exist in systematic trial form.

This is a translational gap with a specific shape. It is not a basic science problem requiring new mechanism discovery. It is not a drug development problem requiring new agents. It is a trial design problem: combining existing or near-approved

components into protocols that engage the full seven-mode failure landscape, in the populations where the framework predicts qualitative benefit.

This paper proposes three specific trials addressing this gap.

1.2 Why these three trials

The three trials selected — BRCA1/2-mutant triple-negative breast cancer, pancreatic ductal adenocarcinoma, microsatellite-stable colorectal cancer — represent three structurally distinct failure-mode patterns and three large unmet-need populations.

BRCA1/2-mutant TNBC has intrinsically favorable biology on Modes 1 and 7 (high tumor mutational burden, platinum-sensitivity reflecting genomic instability that generates antigens). KEYNOTE-522 established that adding pembrolizumab to neoadjuvant chemotherapy improves event-free survival in early TNBC. The framework predicts that systematically engaging Modes 2, 5, 6 alongside the existing KEYNOTE-522 backbone in the BRCA1/2-mutant subset specifically should produce qualitatively better outcomes. This is the population where intrinsic biology and contemporary standard of care have already done most of the work; the remaining unaddressed modes are tractable with FDA-approved components.

Pancreatic ductal adenocarcinoma (PDAC) has the most adverse failure-mode pattern in solid tumor oncology: low Mode 1 (often), severe Mode 3 (desmoplastic stroma), severe Mode 5 (Treg/MDSC-dominated TME), substantial Mode 7 (subclonal heterogeneity in late-stage disease). The autogene cevumeran result (Rojas et al., *Nature* 2023) — HR 0.14 for recurrence in vaccine responders at 3-year follow-up — provides the most striking recent positive signal in any immunotherapy-refractory cancer. The protocol engages Modes 1, 2, 4, 7 through mRNA neoantigen vaccine + atezolizumab + mFOLFIRINOX. The framework identifies Modes 3, 5, 6 as unaddressed and predicts that adding components engaging these modes should extend benefit beyond the vaccine-responder subpopulation.

Microsatellite-stable colorectal cancer (MSS CRC) is the largest single immunotherapy-refractory population in oncology — approximately 85% of all colorectal cancers and roughly 95% of metastatic colorectal cancers, hundreds of thousands of patients per year, essentially zero benefit from single-agent checkpoint blockade. The framework identifies Modes 1, 2, 3, 5 as collectively unfavorable in MSS CRC. The FOLFOX/bevacizumab standard already engages Modes 1, 3, 7 partially through immunogenic chemotherapy and vascular normalization. The framework predicts that adding Mode 2 (intratumoral PAMP) + Mode 4 (checkpoint blockade) + Mode 6 (IL-15 superagonist) to the existing standard could convert MSS CRC from immunotherapy-refractory to immunotherapy-responsive.

These three trials test the framework across the spectrum of intrinsic immunotherapy susceptibility: a moderately responsive cancer (BRCA1/2-mutant TNBC) where the framework predicts conversion of partial responses to durable responses; a paradigmatic refractory cancer (PDAC) where the framework predicts extension of an emerging positive signal; the largest refractory population (MSS CRC) where the framework predicts qualitative conversion of refractory status.

1.3 What "combinatorial-complete" means operationally

A combinatorial-complete protocol in the framework's sense is one that engages 6 or 7 of the seven modes through deliberate intervention design, with each engaged mode supported by mechanistically robust intervention and clinical evidence base for the component agent in at least one indication.

The framework's 0-1 mode scoring methodology (described in Paper 3, Section 9) provides the operational test. A protocol scoring 0.7-1.0 across all seven modes is combinatorial-complete. A protocol scoring 1.0 on one mode and 0.1-0.3 on others is single-mode dominant — the contemporary checkpoint inhibitor pattern. A protocol scoring 0.7-0.9 across five modes and 0 on one mode is combinatorially-incomplete by exactly that dimension — the historical Coley protocol pattern, complete except for Mode 4.

Each trial proposed below targets 0.7-1.0 engagement across all seven modes. The component selections reflect the framework's identification of which intervention category most directly engages each mode, with priority given to FDA-approved agents over investigational ones where both options exist.

1.4 Feasibility assumptions

The trials proposed in this paper are designed to be operationally feasible within current regulatory and clinical infrastructure. Specific assumptions:

Component availability. Every component in every proposed protocol is either FDA-approved for at least one indication or in advanced clinical development (Phase 2/3 trials). No novel-agent development is required.

Manufacturing feasibility. Personalized components (autogene cevumeran-equivalent neoantigen vaccines, autologous TIL preparations) have established manufacturing pathways. Manufacturing timelines (6-8 weeks for personalized vaccines) are integrated into trial schemata.

Multi-sponsor coordination. Several proposed protocols require collaboration between multiple pharmaceutical sponsors. Recent precedents include Genentech-Moderna (autogene cevumeran), Merck-Amgen (MASTERKEY-265), ImmunityBio-Merck (ANKTIVA-pembrolizumab combinations in development). Multi-sponsor academic trials are difficult but not unprecedented.

Regulatory pathway. Each trial design is a phase II proof-of-principle establishing whether combinatorial benefit emerges, followed by phase III if signals warrant. The framework's predictions are sufficiently strong that phase II signals would likely justify rapid phase III progression.

Statistical complexity. Multi-arm factorial designs that fully deconvolve the contribution of each component would require very large sample sizes. The proposed designs use simpler combinatorial-complete-vs-standard comparisons, accepting that individual component contribution cannot be fully disentangled in the proof-of-principle phase.

1.5 Relationship to Paper 3 and to ongoing trials

This paper builds directly on Paper 3 of this series, which establishes the seven-mode framework, the scoring methodology, and the literature integration supporting the framework's predictions. Readers unfamiliar with the framework should consult Paper 3 for the structural foundations.

The trials proposed here are deliberately positioned in spaces where ongoing trials are testing partial combinations but not the full combinatorial-complete protocols. The framework predicts that the ongoing partial-combination trials will produce intermediate benefits — better than single-mode interventions, worse than combinatorial-complete protocols. The proposed trials test the latter directly.

The author is an independent researcher without institutional affiliation or financial conflicts. The trial designs are proposed for adoption by qualified investigators with the institutional infrastructure to implement them. The author's role is structural and analytical, not operational.

1.6 The chemotherapy-sequencing and dosing principle

A central design principle governs all three trials and warrants explicit statement, because it is frequently neglected in combination immunotherapy trials and because neglecting it can defeat the entire combinatorial strategy.

Chemotherapy is neither uniformly immunosuppressive nor uniformly immunostimulatory. Its effect on anti-tumor immunity depends on the agent, the dose, and the timing relative to immune priming. Two opposing effects must be balanced.

On one hand, certain chemotherapies — oxaliplatin, the anthracyclines (doxorubicin), and to a lesser degree the platinums and taxanes — induce *immunogenic cell death*: tumor cells die in a manner that releases tumor antigens and danger-associated molecular signals (calreticulin exposure, HMGB1 and ATP release) that recruit and activate dendritic cells. In this mode, chemotherapy is the *initiator* of an anti-tumor immune response, engaging Modes 1 and 7 and indirectly supporting Mode 2. This immunogenic effect is most pronounced at moderate, well-timed doses.

On the other hand, cytotoxic chemotherapy kills dividing cells indiscriminately, and activated, clonally expanding anti-tumor T cells are among the most rapidly dividing cells in the body during an immune response. High-dose myelosuppressive chemotherapy administered *after* immune priming can therefore destroy the very T cell populations the priming was intended to generate. Lymphodepletion has a place in some adoptive cell therapy protocols (where it creates space for transferred cells), but in the context of an endogenously primed combinatorial-complete response, ill-timed myelosuppression is counterproductive.

The reconciling principle, which governs the trial designs in this paper:

Chemotherapy should be used at immunogenic doses as an immune trigger, with immune priming established before or concurrently with the immunogenic chemotherapy phase, and any necessary myelosuppressive chemotherapy timed to avoid destroying primed and expanding T cell populations.

Concretely, this principle implies: (1) immune-priming components (vaccines, intratumoral danger signals, dendritic-cell engagement) should begin early — at or before the start of immunogenic chemotherapy — rather than being withheld until after chemotherapy is complete; (2) the more immunogenic and less myelosuppressive chemotherapy agents (oxaliplatin, platinum/taxane combinations) are preferred as immune triggers over maximally myelosuppressive regimens where a choice exists; (3) where intensely myelosuppressive chemotherapy is part of the standard backbone, immune components should be sequenced so that the primed response is either established before the myelosuppressive phase and allowed to recover after it, or sustained through it by Mode 6 (persistence) support; and (4) assay-guided or response-guided dosing, which tailors chemotherapy dose to the minimum effective level for a given tumor, is preferable to fixed maximal dosing because it preserves immune competence while still achieving the immunogenic and cytoreductive effects.

This principle is not merely theoretical. It reflects the empirical pattern of the family case that motivates this work (Paper 3, Section 1.5; and Section 2.9 below), in which immune priming was initiated approximately one month before surgery and sustained throughout treatment, and chemotherapy was deliberately assay-guided and low-dose specifically to avoid suppressing the immune response that the immunotherapy components were building. The trial designs below incorporate this sequencing principle explicitly, and the conventional fixed-dose chemotherapy backbones (such as KEYNOTE-522) are treated as starting points to be modified by this principle, not as fixed constraints.

1.7 The modern Coley lineage

The seven-mode framework's combinatorial logic has a direct historical antecedent that should be named explicitly: the mixed bacterial vaccine developed by William B. Coley in the 1890s, and the body of immunotherapy that descends from it.

Coley injected bacterial products — killed cultures of *Streptococcus pyogenes* and *Serratia marcescens*, later termed "Coley's toxins" — into or near tumors, typically inducing high fever and, in a substantial number of documented cases, durable tumor regression. In the language of the present framework, Coley's approach engaged most of the seven modes simultaneously: the bacterial products were potent pathogen-associated molecular patterns (PAMPs) that activated innate immunity and dendritic cells (Mode 2), drove inflammation that disrupted the tumor's physical and suppressive barriers (Modes 3 and 5), exposed and presented tumor antigens released by the inflammatory response (Modes 1 and 7), and — through repeated, sustained dosing over weeks to months — supported persistence of the response (Mode 6). The single mode Coley could not engage was Mode 4, checkpoint blockade, which would not be conceptualized for another century. Coley's protocol was, in the framework's terms, combinatorially near-complete: six of seven modes, missing only the one that did not yet exist as a concept.

This is the framework's central historical insight: the most striking historical immunotherapy results were achieved by a method that engaged the failure-mode landscape broadly, and the field's subsequent century of work has been, in part, a process of rediscovering and refining individual modes that Coley engaged crudely but simul-

taneously.

The trial designs in this paper deliberately incorporate modern molecular descendants of each element of Coley's approach:

- **Intratumoral TLR agonists (poly-ICLC) and other defined danger signals** are the direct modern descendants of Coley's bacterial toxins — purified, characterized, dose-controlled PAMPs delivered into the tumor to trigger the same innate-immune alarm that Coley's crude bacterial preparations produced (Mode 2, with effects on Modes 3 and 5). The in situ vaccination work of Hammerich and colleagues (*Nature Medicine* 2019) is a direct contemporary realization of Coley's intratumoral principle.
- **IL-15 superagonists (N-803/ANKTIVA)** provide the sustained immune engagement that Coley achieved through repeated dosing over weeks (Mode 6), now delivered as a defined cytokine therapeutic.
- **Fever-range and whole-body hyperthermia at 39–40°C**, sustained over multi-hour sessions, is *not* an optional adjunct. The framework — articulated in the companion Neo-Coley v2 paper — treats sustained fever-range thermal stress as one of four load-bearing conditions for durable Coley-type response. Heat-shock proteins released during fever-range thermal stress chaperone tumor antigens into the dendritic-cell cross-presentation pathway; endothelial activation increases lymphocyte trafficking; tumor cells (whose heat tolerance is lower than normal tissue's) are stressed and partially killed. The mechanisms operate cooperatively and depend on the *sustained* nature of the thermal stress. Combinatorial-complete trial protocols are designed to include this component, by hyperthermia equipment where available, by fever-titrated PAMP dosing where it is not.
- **mRNA neoantigen vaccines** provide the precise, tumor-specific antigen targeting (Modes 1, 7) that Coley's approach achieved only nonspecifically through inflammatory antigen release.

Where this paper refers to "modern Coley" components, it means specifically these defined molecular descendants of Coley's intratumoral, sustained, innate-immune-activating approach — chiefly the intratumoral TLR agonist and IL-15 superagonist components, together with sustained fever-range thermal stress at 39–40°C as a load-bearing cross-cutting requirement. The framework's combinatorial-complete protocols can be understood as Coley's broad multi-mode engagement, made molecularly precise, dose-controlled, sustained at fever range, and completed by the addition of the one mode (checkpoint blockade) that Coley's era lacked.

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2. Trial One: Combinatorial-complete immunotherapy for BRCA1/2-mutant triple-negative breast cancer

2.1 Population and rationale

Proposed population: Patients with newly diagnosed, untreated, BRCA1 or BRCA2 germline-mutant triple-negative breast cancer (ER-negative, PR-negative, HER2-

negative; estrogen receptor <1%, progesterone receptor <1%, HER2 IHC 0/1+ or FISH-negative), clinical stage II-III, planned for neoadjuvant therapy and definitive surgery.

Key inclusion criteria: - Germline BRCA1 or BRCA2 mutation confirmed by clinical genetic testing - Triple-negative receptor status per standard criteria - ECOG performance status 0-1 - Adequate organ function for combination therapy - Tumor accessible for biopsy and intratumoral injection - Age 18-75 - No prior systemic therapy for breast cancer

Estimated incidence: Approximately 10-20% of TNBC patients carry germline BRCA1/2 mutations (prevalence varies by population, age at diagnosis, and family history; higher in younger patients and selected populations). With approximately 50,000 new TNBC diagnoses annually in the United States, the eligible population is approximately 5,000-10,000 patients per year. Global eligible population is several times this.

Rationale for combinatorial-complete protocol in this population:

BRCA1/2-mutant TNBC has the most favorable intrinsic biology of any breast cancer subtype for cross-mode combinatorial immunotherapy. The mechanism map:

- **Mode 1 (antigen presentation):** BRCA1/2-mutant tumors carry homologous recombination deficiency that generates genomic instability and elevated mutational and neoantigen load relative to homologous-recombination-proficient tumors, producing more neoantigens for presentation. MHC class I expression is generally intact in early-stage disease.
- **Mode 7 (heterogeneity):** Platinum sensitivity in BRCA1/2-mutant TNBC reflects genomic instability that produces diverse mutation profiles — favorable for broad neoantigen recognition but also presenting subclonal heterogeneity that combinatorial-complete protocols are designed to address.
- **Mode 4 (exhaustion):** PD-L1 expression occurs in approximately 35-40% of TNBC tumors, with PD-L1-positive TNBC showing greater checkpoint inhibitor benefit. KEYNOTE-522 established pembrolizumab + chemotherapy benefit in early TNBC regardless of PD-L1 status.

The remaining modes are not adequately addressed by contemporary standard of care:

- **Mode 2 (T cell priming):** Pembrolizumab + chemotherapy provides indirect priming through immunogenic cell death but does not deliberately engage dendritic cell expansion or activation. The Bhardwaj 2020 *Nature Cancer* result (Flt3L augments immune responses to NY-ESO-1 vaccine) demonstrates the magnitude of benefit available from explicit Mode 2 engagement.
- **Mode 3 (physical exclusion):** TNBC tumors vary in T cell infiltration; the desmoplastic stromal component is less severe than PDAC but is present, particularly in basal-like TNBC.
- **Mode 5 (active suppression):** Tregs, MDSCs, and M2-polarized macrophages infiltrate TNBC, particularly in PD-L1-negative tumors. Tanaka and Sakaguchi 2017 (*Cell Research*) and the 2025 Nobel Prize for Treg biology highlight the centrality of Mode 5 in TNBC and other adverse tumor microenvironments.

- **Mode 6 (persistence):** Pembrolizumab + chemotherapy provides fixed-duration treatment without explicit memory T cell support. KEYNOTE-942 (Weber et al., *Lancet* 2024) demonstrated that adding personalized mRNA neoantigen vaccine — engaging Modes 1, 2, 7 with substantial Mode 6 effect — to pembrolizumab in adjuvant melanoma improved RFS with HR 0.561.

The framework predicts that adding deliberate Mode 2, 5, and 6 engagement to the immunogenic-chemotherapy/checkpoint backbone in BRCA1/2-mutant TNBC — and, critically, sequencing it so that immune priming precedes and is protected from cytoreductive chemotherapy (Section 1.6) — will produce qualitatively better outcomes than the conventional KEYNOTE-522 regimen, in which full-dose chemotherapy is applied throughout and immunity is engaged only through checkpoint blockade as a byproduct.

2.2 Proposed intervention: Six-mode combinatorial-complete protocol with immune-first sequencing

Trial name (proposed): BREAKTHROUGH-1 (Broadened Response by Engaging All seven modes — combinatorial-complete immunotherapy in BRCA1/2-mutant triple-negative breast cancer).

Design principle applied (per Section 1.6): The intervention is sequenced so that immune priming is established *before and during* the immunogenic chemotherapy phase, the immunogenic and less-myelosuppressive chemotherapy (platinum/taxane) serves as the primary immune trigger, and the more myelosuppressive anthracycline/cyclophosphamide phase is repositioned and de-emphasized so that it does not destroy the primed and expanding T cell population. This is the key departure from a conventional KEYNOTE-522 backbone with immunotherapy simply layered on top.

Intervention components and their roles:

1. **mRNA personalized neoantigen vaccine** (autogene cevumeran-equivalent, up to 20 neoantigens per patient) — engages Modes 1, 2, 6, 7 via dendritic-cell activation, broad neoantigen exposure, and sustained antigen stimulation. *Initiated early*, as soon as the vaccine is manufactured from the diagnostic biopsy.
2. **Intratumoral poly-ICLC** (TLR3 agonist; the modern-Coley danger-signal component) — engages Modes 2 and 3 via direct dendritic-cell activation and inflammatory disruption of the excluded/suppressive microenvironment. *Begins at the start of treatment*, into the intact primary tumor, where it can prime against the full antigen repertoire before cytoreduction.
3. **Carboplatin + paclitaxel** (immunogenic, moderately myelosuppressive) — engages Modes 1 and 7 via immunogenic cell death; serves as the *immune trigger* concurrent with immune priming, at standard or assay-/response-guided dose.
4. **Pembrolizumab** (anti-PD-1) — engages Mode 4, protecting the newly primed T cells from exhaustion; begins concurrently with priming and the immunogenic chemotherapy.
5. **N-803 IL-15 superagonist** (ANKTIVA; the modern-Coley persistence component) — engages Mode 6 via memory T cell support, sustaining the primed response, including through any subsequent myelosuppressive phase and into the adjuvant period.

6. **Sustained fever-range thermal stress at 39–40°C** (whole-body hyperthermia where institutional equipment exists, or fever-titrated poly-ICLC dosing where it does not) — a *load-bearing cross-cutting component* of the Neo-Coley framework that simultaneously augments antigen presentation (Mode 1, via heat-shock-protein-mediated cross-presentation to dendritic cells), enhances T-cell trafficking (Mode 3, via the HSP90α–integrin pathway characterized by the Repasky and Evans laboratories), supports innate immune engagement (Mode 2, via amplification of the PAMP response), and contributes to broad antigen release (Mode 7, via heat-induced immunogenic tumor cell death). The framework's prediction is that the same combinatorial component set without sustained fever-range thermal stress produces qualitatively reduced responses — this component is required for the protocol to qualify as combinatorially complete in the Neo-Coley sense.

Sequence (intervention arm):

Priming window (weeks 1-3, before cytoreductive chemotherapy dominates): intratumoral poly-ICLC into the intact tumor + first vaccine doses (once manufactured) + pembrolizumab initiation + **weekly whole-body hyperthermia (39–40°C, sustained 2–4 hours per session) or fever-titrated poly-ICLC dosing**. The goal is to establish dendritic-cell activation and begin neoantigen priming against the full, untreated tumor antigen repertoire, with sustained fever-range thermal stress augmenting cross-presentation and T-cell trafficking from week 1.

Immunogenic trigger phase (weeks 3-15): carboplatin + paclitaxel + continued pembrolizumab + continued vaccine dosing + intratumoral poly-ICLC (tapering as the tumor regresses) + N-803 + **continued weekly hyperthermia where feasible alongside chemotherapy cycles**. The platinum/taxane provides immunogenic cell death that amplifies antigen availability while the primed immune response is already active. This phase is moderately but not maximally myelosuppressive.

Conditional anthracycline phase (weeks 15-23, modified): the doxorubicin/cyclophosphamide (AC) component of the conventional KEYNOTE-522 backbone is the most myelosuppressive element and the greatest threat to primed T cells. Per the Section 1.6 principle, this phase is handled in one of two pre-specified ways, tested as a secondary randomization or by cohort: (a) **retained but protected** — AC given with continued N-803 (IL-15) support to sustain the memory T cell pool through the myelosuppressive window, with vaccine boosting resumed after recovery; or (b) **reduced/omitted** — for patients with strong on-treatment response (assessed by imaging and/or circulating tumor DNA after the immunogenic trigger phase), the AC phase is reduced or omitted to preserve the established immune response, an approach consistent with the assay-/response-guided dosing principle and with emerging de-escalation strategies in highly responsive TNBC.

Surgery: definitive surgery follows, with pathologic complete response (pCR) assessment, timed to allow immune recovery (minimum washout from last intratumoral injection and from myelosuppressive chemotherapy).

Adjuvant phase (post-surgery, to complete ~1 year of immunotherapy): pembrolizumab + N-803 maintenance + mRNA vaccine maintenance dosing + **monthly**

hyperthermia maintenance where feasible, sustaining the primed and surgically-debulked-state immune response (Mode 6).

Comparator arm: Standard KEYNOTE-522 protocol (carboplatin + paclitaxel + pembrolizumab → doxorubicin/cyclophosphamide + pembrolizumab → surgery → adjuvant pembrolizumab), which administers full-dose myelosuppressive chemotherapy throughout and does not deliberately establish immune priming before cytoreduction.

The central design contrast is therefore not only *which* modes are engaged but *when*: the intervention arm establishes immunity first and uses immunogenic chemotherapy as a trigger while protecting the primed response from myelosuppression, whereas the comparator applies fixed full-dose chemotherapy with checkpoint blockade and assesses immunity only as a byproduct.

2.3 Mode-by-mode coverage analysis

Mode	Engagement	Component(s)	Coverage score
1: Antigen presentation	Strong	Chemotherapy (immunogenic cell death) + mRNA vaccine + intratumoral poly-ICLC	0.95
2: T cell priming	Strong	Intratumoral poly-ICLC + mRNA vaccine	0.90
3: Physical exclusion	Moderate	Intratumoral delivery + inflammatory disruption	0.70
4: T cell exhaustion	Strong	Pembrolizumab	1.00
5: Active suppression	Moderate	Inflammatory disruption + chemotherapy MDSC depletion	0.60
6: Persistence	Strong	N-803 IL-15 superagonist + sustained vaccine dosing	0.95
7: Tumor heterogeneity	Strong	Chemotherapy + mRNA polyepitope (up to 20) + intratumoral inflammation	0.90

Total combinatorial-complete coverage achieved across all seven modes, with

Mode 3 and Mode 5 the relative weak points. The framework's prediction is that this coverage pattern should produce qualitatively better outcomes than the KEYNOTE-522 standard (which scores 0.5-0.6 on Modes 1, 4, 7 and 0.1-0.3 on the others).

2.4 Endpoints

Primary endpoint: Pathologic complete response (pCR; ypT0/Tis ypN0) at definitive surgery. KEYNOTE-522 demonstrated pCR rates of 64.8% with pembrolizumab + chemotherapy vs 51.2% with chemotherapy alone in unselected TNBC. The framework predicts the combinatorial-complete protocol will achieve pCR rates of 80% or higher in BRCA1/2-mutant TNBC specifically.

Key secondary endpoints: - Event-free survival (EFS) at 3 and 5 years - Distant metastasis-free survival (DMFS) - Overall survival (OS) - pCR rates stratified by PD-L1 status - Quality-of-life measures - Safety and toxicity (immune-related adverse events, intratumoral injection-related events, hematologic toxicity)

Exploratory endpoints (correlative science to test the framework): - Tumor-infiltrating lymphocyte density and phenotype at baseline, mid-treatment biopsy, and surgical specimen - Vaccine-induced neoantigen-specific T cell response quantification (ELISpot, MHC tetramer) - HLA class I expression dynamics (baseline vs surgical specimen) - Subclonal neoantigen tracking (whole-exome sequencing pre/post) - TCR clonality and persistence (peripheral blood and tumor) - Treg, MDSC, and M2-macrophage quantification in tumor microenvironment - Memory T cell phenotype in peripheral blood - Circulating tumor DNA (ctDNA) dynamics

The exploratory endpoints test the framework's mode-specific mechanisms. If combinatorial-complete protocol produces pCR rate advantage, the correlative data should show that the advantage correlates with successful engagement of the multiple modes — vaccine-induced T cell responses (Mode 2), sustained T cell persistence (Mode 6), broad neoantigen targeting (Mode 7), etc.

2.5 Statistical design

Trial design: Randomized phase II, 2:1 randomization (intervention:control), with phase III expansion conditional on phase II results.

Sample size estimation: Assuming standard KEYNOTE-522 pCR rate of 65% in BRCA1/2-mutant TNBC subset and framework-predicted combinatorial-complete pCR rate of 82%, with two-sided alpha 0.05 and power 0.85: - Intervention arm: n = 120 - Control arm: n = 60 - Total phase II: n = 180

This sample size is feasible within a 3-year enrollment period across 15-20 high-volume breast cancer centers.

Phase III expansion design: If phase II demonstrates pCR advantage of ≥12 percentage points with appropriate safety profile, automatic expansion to phase III with EFS primary endpoint, sample size approximately 400-500 per arm.

2.6 Biomarker strategy

Pre-specified biomarker hypotheses:

1. **pCR advantage will correlate with vaccine-induced neoantigen T cell response magnitude.** This tests the framework's Mode 2 + 6 + 7 prediction directly. If patients with strong vaccine responses (>3-fold expansion of neoantigen-specific T cells) show pCR rate of 90%+ while non-responders show pCR rates similar to control, the framework's mechanism is validated.
2. **Patients with intact baseline MHC class I and absent HLA LOH will show greater combinatorial benefit than those with antigen presentation defects.** This tests the framework's Mode 1 dependency. Pre-treatment HLA LOH assessment is straightforward with current sequencing platforms.
3. **Patients with high baseline Treg/MDSC infiltration will show smaller combinatorial benefit than those with low baseline Treg/MDSC.** This tests the framework's Mode 5 hypothesis and identifies where additional Mode 5 components might be needed in future protocols.
4. **Sustained T cell persistence (measured 3-6 months post-treatment) will correlate with EFS, independent of pCR status.** This tests Mode 6 as a distinct mechanism from initial response.

These pre-specified hypotheses make the trial a mechanistic test of the framework, not merely an efficacy comparison.

2.7 Safety considerations

Anticipated toxicity profile:

- **Carboplatin + paclitaxel:** Standard chemotherapy toxicity (cytopenias, neuropathy, hypersensitivity). Well-characterized.
- **Pembrolizumab:** Standard immune-related adverse events (thyroid dysfunction, pneumonitis, colitis, hepatitis, etc.) at rates established in KEYNOTE-522 and other trials.
- **mRNA vaccine (autogene cevumeran-equivalent):** Local injection-site reactions, mild systemic flu-like symptoms; cytokine-mediated effects within established safety profile from Rojas 2023 and Weber 2024.
- **Intratumoral poly-ICLC:** Local pain, transient fever, injection-site inflammation; rare systemic cytokine effects. Established safety profile from multiple in situ vaccination trials.
- **N-803 (ANKTIVA):** Well-established safety profile from QUILT-3.032 NMIBC trials; primarily mild-moderate constitutional symptoms, transient cytokine effects. No new-class toxicity.

Anticipated combination toxicity concerns:

- **Overlapping immune-related adverse events** with checkpoint blockade + IL-15 superagonist + intratumoral PAMP could increase grade 3+ irAE rates. Pre-specified safety stopping rules (e.g., grade 3+ irAE rate >25% triggers protocol amendment).

- **Cytokine release syndrome** is theoretically possible with combination IL-15 + PAMP + checkpoint, though not observed clinically with individual components at proposed doses. Pre-specified CRS monitoring per established criteria.
- **Wound healing impact** from anti-angiogenic effects, immune activation, and proximity to surgery requires pre-specified surgery timing (4-week washout from last intratumoral injection; 2-week washout from last pembrolizumab dose).

Independent Data Safety Monitoring Board with frequent (every 20 patients) interim safety analysis. Pre-specified futility analysis at 60 patients.

2.8 Feasibility analysis

Component availability:

Component	Status	Manufacturer
Carboplatin + paclitaxel	FDA-approved, generic	Multiple
Pembrolizumab (Keytruda)	FDA-approved for early TNBC	Merck
mRNA personalized neoantigen vaccine	Late-phase clinical (autogene cevumeran in PDAC; mRNA-4157 in melanoma)	Genentech/BioNTech; Moderna/Merck
Poly-ICLC (Hiltonol)	Investigational; used in multiple trials including in situ vaccination	Oncovir
N-803 (ANKTIVA, nogapendekin alfa inbakicept)	FDA-approved for NMIBC (April 22, 2024)	ImmunityBio

Multi-sponsor coordination required: Merck (pembrolizumab) + Genentech/Moderna (mRNA vaccine) + ImmunityBio (ANKTIVA) + Oncovir (poly-ICLC) + academic investigator network. Precedent exists for such coordination (KEYNOTE-942 was Merck-Moderna; autogene cevumeran trials are Genentech-BioNTech).

Manufacturing logistics: Personalized mRNA vaccine requires 6-8 weeks manufacturing from surgical biopsy. Trial schema integrates initial KEYNOTE-522 backbone (8-12 weeks) with vaccine manufacturing, allowing combined protocol from week 8-12 onward. Manufacturing capacity is the rate-limiting feasibility constraint, currently scaling rapidly.

Estimated trial cost: $15-30 million for phase II (180 patients, 15-20 sites, 3-year enrollment, 5-year follow-up). Cost-effective relative to potential clinical impact if the framework's prediction proves correct.

Estimated trial duration: 8-10 years total (3 years phase II enrollment + 2 years phase II follow-up + 2-3 years phase III if triggered + ongoing follow-up).

2.9 Personal context disclosure

The author of this paper has disclosed in Paper 3 (Section 1.5) personal involvement in cancer immunotherapy as a family caregiver, specifically a 2011 case of Stage IIA high-grade BRCA1-mutated triple-negative invasive ductal carcinoma with metaplastic features in his wife, with sustained 15-year recurrence-free survival following multi-mode combinatorial treatment including Coley vaccine, fever-range whole-body hyperthermia, tumor-antigen vaccine, and assay-guided low-dose platinum chemotherapy.

This personal context is acknowledged. The trial design above stands on its mechanistic merits independent of the personal case. The framework's structural arguments are made from published literature; the proposed trial addresses a translational gap identified by that framework; the BRCA1/2-mutant TNBC population is the natural test population because it has the most favorable intrinsic biology for combinatorial-complete benefit, not because of the author's personal connection to it.

However, the personal context provides motivational coherence. The author's family experience suggests that combinatorial multi-mode treatment in this population can produce durable benefit. The proposed trial would test whether contemporary combinatorial protocols using FDA-approved components achieve similar durable benefit in a controlled clinical setting. The author has direct interest in this trial being run, openly disclosed, and stands to gain nothing financially from any specific outcome.

The author's middle daughter Gabriella is 23 years old and BRCA1-mutation positive. If BREAKTHROUGH-1 (or equivalent combinatorial-complete protocols) had been clinical standard of care in 2011, treatment uncertainty for high-risk BRCA1 carriers like Gabriella in 2026-2040 would be substantially reduced. This is the personal stake, openly stated.

2.10 Why this trial should be run now

The components exist. The mechanistic rationale is established by Paper 3 of this series and by extensive literature support for each individual component. The population is well-defined and large enough to enroll. The endpoints are clinically meaningful and statistically achievable. The infrastructure exists at major academic breast cancer centers.

The translational gap is operational rather than scientific. The trial requires multi-sponsor coordination, dedicated investigator leadership, and institutional commitment to test a combinatorial-complete protocol against the existing single-mode-extension paradigm. None of these are easy. All are achievable.

The framework predicts that BREAKTHROUGH-1 will demonstrate pCR rate improvement of 15-20 percentage points over KEYNOTE-522 standard in BRCA1/2-mutant TNBC, with corresponding EFS and OS benefits. If the framework is wrong, the trial will show this empirically and the field can move on. If the framework is correct, BREAKTHROUGH-1 will establish combinatorial-complete protocols as the new standard for BRCA1/2-mutant TNBC and provide structural validation for similar protocols across the immunotherapy-refractory cancer landscape.

The trial should be run. The framework predicts it will work.

3. Trial Two: Extended combinatorial-complete protocol for resectable pancreatic ductal adenocarcinoma

3.1 Population and rationale

Proposed population: Patients with resectable or borderline-resectable pancreatic ductal adenocarcinoma (PDAC), planned for surgical resection with curative intent, eligible for adjuvant systemic therapy.

Key inclusion criteria: - Histologically or cytologically confirmed PDAC - Resectable or borderline-resectable disease per NCCN criteria - Planned R0/R1 surgical resection - ECOG performance status 0-1 - Adequate organ function for combination therapy including mFOLFIRINOX - Tumor tissue available for neoantigen identification and vaccine manufacturing - Age 18-75 - No prior systemic therapy for PDAC

Estimated incidence: Approximately 66,000 new PDAC diagnoses annually in the United States. Approximately 15-20% present with resectable or borderline-resectable disease — roughly 10,000-13,000 patients per year eligible for adjuvant-setting trials. PDAC is the third leading cause of cancer death in the United States and is projected to become the second within the decade.

Rationale for extended combinatorial-complete protocol:

PDAC is the paradigmatic immunotherapy-refractory cancer. Single-agent checkpoint blockade is essentially inactive. The framework attributes this refractoriness to a uniquely adverse multi-mode failure pattern:

- **Mode 1 (antigen presentation):** Variable; PDAC has relatively low tumor mutational burden compared to melanoma or NSCLC, but harbors recurrent driver mutations (KRAS, TP53, CDKN2A, SMAD4) and patient-specific neoantigens suitable for vaccine targeting (demonstrated by autogene cevumeran).
- **Mode 3 (physical exclusion):** Severe. PDAC's defining histological feature is dense desmoplastic stroma — cancer-associated fibroblasts, extracellular matrix, and abnormal vasculature that physically and functionally exclude T cells. Kieffer et al. (*Cancer Discovery* 2020) characterized the CAF subsets that mediate immunosuppression and immunotherapy resistance. This is PDAC's dominant failure mode.
- **Mode 5 (active suppression):** Severe. The PDAC tumor microenvironment is dominated by regulatory T cells, myeloid-derived suppressor cells, and tumor-associated macrophages. Adenosine-mediated suppression (CD73/CD39/A2A axis) is prominent. Tregs and MDSCs are recruited early in PDAC pathogenesis.
- **Mode 7 (heterogeneity):** Substantial in advanced disease; less limiting in resectable early disease where the autogene cevumeran trial demonstrated benefit.

The autogene cevumeran result and what it leaves unaddressed:

Rojas et al. (*Nature* 2023) reported a phase I trial of adjuvant autogene cevumeran —

an individualized mRNA neoantigen vaccine (up to 20 neoantigens per patient) based on uridine mRNA-lipoplex nanoparticles — given sequentially with atezolizumab (anti-PD-L1) and mFOLFIRINOX chemotherapy in resected PDAC. The result was striking: in the 8 of 16 patients who developed vaccine-induced neoantigen-specific T cell responses, recurrence-free survival was dramatically prolonged (hazard ratio 0.14) compared to non-responders at extended follow-up. Guasp et al. (*Nature* 2025) reported that these vaccine-induced T cells persist as long-lived effector cells, in some cases detectable years after vaccination.

This is the most significant positive immunotherapy signal ever reported in PDAC. It demonstrates that the immunotherapy-refractory status of PDAC can be overcome — at least in the subset of patients who mount vaccine responses.

The framework analysis of the autogene cevumeran protocol:

Mode	Engagement by autogene cevumeran protocol	Coverage
1	mRNA neoantigen vaccine	0.85
2	mRNA vaccine (lipoplex provides adjuvant/DC effect)	0.70
3	Not directly addressed	0.10
4	Atezolizumab	0.90
5	Not directly addressed	0.10
6	Sustained vaccine dosing; T cell persistence demonstrated	0.75
7	Polyepitope vaccine (up to 20 neoantigens)	0.80

The autogene cevumeran protocol achieves combinatorial benefit on Modes 1, 2, 4, 6, 7 while leaving Modes 3 and 5 — PDAC's two dominant failure modes — essentially unaddressed.

This is the critical insight. The autogene cevumeran result is achieved despite leaving PDAC's defining failure modes (stromal exclusion, active suppression) unaddressed. Only patients who happen to mount strong vaccine responses despite the hostile stromal/suppressive microenvironment benefit. The framework predicts that adding Mode 3 and Mode 5 engagement to the autogene cevumeran backbone will extend benefit to a larger fraction of patients by overcoming the stromal exclusion and active suppression that currently limit vaccine response to the favorable-microenvironment subset.

3.2 Proposed intervention: Seven-mode extended protocol

Trial name (proposed): BREAKTHROUGH-2 (Broadened Response by Engaging All seven modes — adding stromal modification and suppression reversal to the vaccine-checkpoint backbone in pancreatic cancer).

For clarity in this paper, referred to as **BREAKTHROUGH-2** or the **extended autogene cevumeran protocol**.

Intervention arm (building on the autogene cevumeran backbone):

Following surgical resection, sequential adjuvant therapy:

1. **Atezolizumab** (1200 mg IV every 3 weeks) — engages Mode 4 (PD-L1 blockade); retained from autogene cevumeran protocol
2. **Autogene cevumeran-equivalent mRNA neoantigen vaccine** (up to 20 neoantigens, IV per established dosing schedule) — engages Modes 1, 2, 6, 7; retained from original protocol
3. **mFOLFIRINOX** (modified folinic acid, fluorouracil, irinotecan, oxaliplatin) — engages Modes 1, 7 via immunogenic cell death; retained from original protocol
4. **NEW — Stromal-modifying agent for Mode 3:** Either anti-VEGF (bevacizumab) for vascular normalization, or a focal adhesion kinase (FAK) inhibitor, or losartan (angiotensin inhibition shown to reduce PDAC stromal density and improve drug delivery). Bevacizumab is the most clinically established; the framework's Mode 3 engagement via vascular normalization is supported by the IMbrave150 result (Finn et al., *NEJM* 2020) demonstrating anti-VEGF + checkpoint synergy in hepatocellular carcinoma.
5. **NEW — Adenosine axis inhibitor for Mode 5:** A CD73 inhibitor (oleclumab) or A2A receptor antagonist. Bendell et al. (*Cancer Immunology, Immunotherapy* 2023) established the safety profile of oleclumab in combination immunotherapy. The CD73/adenosine axis is a dominant Mode 5 mechanism in PDAC.
6. **NEW — IL-15 superagonist for enhanced Mode 6:** N-803 (ANKTIVA) to amplify and sustain the vaccine-induced T cell response that the autogene cevumeran data show is the critical determinant of benefit. The framework predicts that strengthening Mode 6 will convert more patients into durable vaccine responders.

Comparator arms (3-arm design): - Arm A: Standard adjuvant mFOLFIRINOX alone (current standard of care for resected PDAC) - Arm B: Autogene cevumeran protocol (atezolizumab + mRNA vaccine + mFOLFIRINOX) — replicating the Rojas 2023 regimen - Arm C: Extended seven-mode protocol (autogene cevumeran backbone + stromal agent + adenosine inhibitor + IL-15 superagonist)

The 3-arm design directly tests the framework's central prediction: that adding Mode 3 + Mode 5 + enhanced Mode 6 engagement (Arm C) to the autogene cevumeran backbone (Arm B) extends benefit beyond what the backbone alone achieves, and that both immunotherapy arms exceed chemotherapy alone (Arm A).

Sequencing principle (per Section 1.6) — already validated by the backbone: Notably, the autogene cevumeran protocol on which this trial builds already embodies the immune-first sequencing principle. In the Rojas 2023 regimen, atezolizumab and the mRNA vaccine were administered *first*, with mFOLFIRINOX chemotherapy deliberately sequenced *afterward*, specifically so that cytotoxic chemotherapy would not blunt the vaccine-induced T cell response during its critical priming and expansion window. This sequencing — immune priming established before cytoreductive chemotherapy — is widely credited as one reason the trial succeeded where prior chemotherapy-first immunotherapy attempts in PDAC failed. The extended protocol (Arm C) preserves this immune-first sequence and adds the Mode 3 stromal-modifying agent and Mode 5 adenosine inhibitor *early*, alongside the priming phase, so that stromal barriers and adenosine-mediated suppression are reduced *while* the vaccine is priming rather than after. The IL-15 superagonist (Mode 6) is sustained through and beyond the chemotherapy phase to protect the primed response — the same logic

applied in Trial 1. This trial thus serves as partial validation that the Section 1.6 sequencing principle is not merely theoretical: the one combinatorial protocol that has produced a striking positive signal in PDAC is one that already sequenced immunity before chemotherapy.

3.3 Mode-by-mode coverage analysis (Arm C)

Mode	Engagement	Component(s)	Coverage score
1: Antigen presentation	Strong	mRNA vaccine + mFOLFIRINOX (immunogenic cell death)	0.85
2: T cell priming	Moderate-strong	mRNA vaccine lipoplex adjuvant effect	0.70
3: Physical exclusion	Moderate	Stromal-modifying agent (anti-VEGF/FAK inhibitor/losartan)	0.65
4: T cell exhaustion	Strong	Atezolizumab	0.90
5: Active suppression	Moderate	CD73/A2A inhibitor + chemotherapy MDSC effects	0.65
6: Persistence	Strong	N-803 IL-15 superagonist + sustained vaccine	0.90
7: Tumor heterogeneity	Strong	mRNA polyepitope + chemotherapy	0.80

Arm C achieves combinatorial-complete coverage across all seven modes, with the previously-unaddressed Modes 3 and 5 raised from ~0.10 (autogene cevumeran alone) to ~0.65. The framework predicts this conversion from five-mode to seven-mode coverage will substantially increase the fraction of patients achieving durable vaccine-induced responses.

3.4 Endpoints

Primary endpoint: Recurrence-free survival (RFS) at 18 months, comparing Arm C to Arm A (chemotherapy alone) as the registrational comparison, with Arm B (autogene cevumeran backbone) as the key mechanistic comparator.

The Rojas 2023 data showed median RFS not reached in vaccine responders versus 13.4 months in non-responders. The framework predicts Arm C will increase the vaccine-responder fraction from approximately 50% (autogene cevumeran) to 70-80%, with corresponding improvement in overall RFS.

Key secondary endpoints: - Overall survival (OS) at 2, 3, and 5 years - Vaccine response rate (proportion developing neoantigen-specific T cell responses) — directly comparing Arm B vs Arm C to test whether Mode 3/5/6 enhancement increases vaccine responder fraction - RFS in vaccine responders vs non-responders within each arm - Surgical and treatment-related morbidity - Safety and toxicity

Exploratory endpoints (correlative science to test the framework): - Stromal density and CAF phenotype dynamics (baseline biopsy vs on-treatment imaging/biopsy) — tests Mode 3 engagement - Intratumoral CD8 T cell infiltration depth and distribution — tests whether Mode 3 modification permits T cell penetration of stroma - Treg, MDSC, and adenosine-pathway marker quantification — tests Mode 5 engagement - Vaccine-induced neoantigen-specific T cell magnitude, phenotype, and persistence — tests Modes 2, 6 - Circulating tumor DNA dynamics as early recurrence marker - Single-cell analysis of tumor microenvironment evolution

3.5 Statistical design

Trial design: Randomized phase II, three arms, 1:1:1 randomization, with adaptive expansion of the most promising arm to phase III.

Sample size estimation: Assuming 18-month RFS of 35% with chemotherapy alone (Arm A, consistent with historical adjuvant mFOLFIRINOX data), 50% with autogene cevumeran backbone (Arm B), and framework-predicted 65% with extended seven-mode protocol (Arm C): - Per arm: n = 70 - Total phase II: n = 210

This sample size provides 80% power to detect the Arm C vs Arm A difference at two-sided alpha 0.05, with the Arm B vs Arm C comparison powered as a key secondary mechanistic analysis.

Phase III expansion: If Arm C demonstrates RFS advantage over both comparators with acceptable safety, expansion to phase III with OS primary endpoint.

3.6 Biomarker strategy

Pre-specified biomarker hypotheses:

1. **The vaccine-responder fraction will be higher in Arm C than Arm B.** This is the central test of the framework's prediction. If adding Mode 3/5/6 engagement increases the proportion of patients who mount vaccine responses (e.g., from 50% to 70%+), the framework's hypothesis — that stromal exclusion and active suppression limit vaccine response in the autogene cevumeran protocol — is validated.
2. **Mode 3 engagement (reduced stromal density, increased T cell infiltration depth) will correlate with vaccine response in Arm C.** Patients whose stroma is successfully modified should show greater T cell penetration and greater vaccine-induced response.
3. **Baseline adenosine-pathway activity (CD73 expression, adenosine levels) will predict differential benefit from the CD73/A2A inhibitor component.**

Patients with high baseline adenosine-mediated suppression should benefit more from Mode 5 engagement.

4. **IL-15 superagonist will increase vaccine-induced T cell persistence (measured 6-12 months post-treatment), correlating with RFS.** Tests the Mode 6 contribution.

3.7 Safety considerations

Anticipated toxicity profile:

The autogene cevumeran protocol components (atezolizumab + mRNA vaccine + mFOLFIRINOX) have established safety from Rojas 2023. The added components introduce additional considerations:

- **Stromal-modifying agent:** If bevacizumab, standard anti-VEGF toxicity (hypertension, proteinuria, thrombosis, impaired wound healing — particularly relevant in post-surgical setting, requiring careful timing). If FAK inhibitor or losartan, generally milder profiles.
- **CD73/A2A inhibitor (oleclumab):** Established safety profile from Bendell 2023; primarily mild constitutional and immune-related effects.
- **N-803 (ANKTIVA):** Established safety from QUILT-3.032; mild-moderate constitutional symptoms.

Combination toxicity concerns: - Post-surgical wound healing with anti-VEGF requires careful timing (minimum 4-6 weeks post-surgery before bevacizumab initiation). - mFOLFIRINOX is itself substantially toxic; adding immunotherapy components requires careful monitoring of overlapping toxicities (diarrhea, fatigue, cytopenias). - Pre-specified safety stopping rules and independent DSMB with frequent interim analysis.

The three-arm design provides internal safety comparison: Arm C toxicity is directly comparable to Arm B (autogene cevumeran backbone) and Arm A (chemotherapy alone), allowing precise attribution of any added toxicity to the Mode 3/5/6 components.

3.8 Feasibility analysis

Component availability:

Component	Status	Manufacturer
mFOLFIRINOX	FDA-approved, generic	Multiple
Atezolizumab (Tecentriq)	FDA-approved	Genentech/Roche
Autogene cevumeran	Phase II (IMCODE003, NCT05968326)	Genentech/BioNTech
Bevacizumab (Avastin)	FDA-approved	Genentech/Roche
Oleclumab (CD73 inhibitor)	Phase II investigational	AstraZeneca

Component	Status	Manufacturer
N-803 (ANKTIVA)	FDA-approved for NMIBC	ImmunityBio

Multi-sponsor coordination: Genentech/Roche owns atezolizumab, bevacizumab, and partners with BioNTech on autogene cevumeran — a substantial fraction of the protocol is within one corporate family, simplifying coordination. AstraZeneca (oleclumab) and ImmunityBio (ANKTIVA) would need to participate. The Genentech-centric backbone is a feasibility advantage.

Manufacturing logistics: Autogene cevumeran requires 6-8 weeks manufacturing from surgical specimen. The adjuvant setting accommodates this timeline (chemotherapy can begin while vaccine is manufactured, with vaccine integrated once available — exactly as in the Rojas 2023 protocol).

Estimated trial cost: $20-35 million for phase II (210 patients, 3-arm, 4-year enrollment, 5-year follow-up).

Estimated trial duration: 9-11 years total.

3.9 Why this trial should be run now

The autogene cevumeran result is the most important positive immunotherapy signal ever reported in pancreatic cancer. It demonstrates that PDAC's immunotherapy-refractory status can be overcome. But it overcomes that status in only the subset of patients who mount vaccine responses despite PDAC's hostile stromal and suppressive microenvironment.

The framework makes a specific, testable prediction: the patients who fail to respond to autogene cevumeran fail because the stromal exclusion (Mode 3) and active suppression (Mode 5) that define PDAC's microenvironment prevent vaccine-induced T cells from functioning. Adding deliberate Mode 3 and Mode 5 engagement should convert non-responders to responders, extending the dramatic benefit currently limited to half of treated patients.

This is the single most consequential combinatorial extension the framework identifies in any cancer, because it builds on the strongest existing positive signal in the most lethal common cancer. The components exist. The backbone protocol is already in phase II. The added components are FDA-approved or in advanced development. The trial requires combining them — a trial design step, not a discovery step.

The framework predicts that BREAKTHROUGH-2 will increase the vaccine-responder fraction from approximately 50% to 70-80%, with corresponding RFS and OS benefits, converting autogene cevumeran from a therapy that helps half of treated patients into one that helps the majority.

The trial should be run. The framework predicts it will extend the most important recent advance in pancreatic cancer to far more patients.

4. Trial Three: Multi-mode protocol for microsatellite-stable colorectal cancer

4.1 Population and rationale

Proposed population: Patients with microsatellite-stable (MSS) metastatic colorectal cancer, planned for first-line systemic therapy.

Key inclusion criteria: - Histologically confirmed colorectal adenocarcinoma - Microsatellite-stable / mismatch-repair-proficient (MSS/pMMR) status confirmed by IHC or PCR - Metastatic disease (stage IV) or unresectable locally advanced disease - Candidate for first-line FOLFOX + bevacizumab - At least one tumor lesion accessible for intratumoral injection (liver, lymph node, or other accessible metastasis) - ECOG performance status 0-1 - Adequate organ function - Age 18-75

Estimated incidence: Colorectal cancer is among the most common cancers worldwide, with approximately 150,000 new diagnoses annually in the United States. Approximately 85% of all colorectal cancers are MSS/pMMR; in the metastatic setting the proportion is higher, with MSI-high/dMMR comprising only about 5% of metastatic CRC and MSS/pMMR comprising approximately 95%. This represents the largest single immunotherapy-refractory population in oncology — well over 100,000 patients per year in the United States alone, and the majority of the millions diagnosed globally.

Rationale — the largest translational opportunity:

MSS colorectal cancer is essentially completely refractory to single-agent checkpoint blockade. While MSI-high colorectal cancer (approximately 5% of metastatic cases) responds dramatically to PD-1 blockade — so much so that pembrolizumab is now first-line standard for MSI-high metastatic CRC — MSS colorectal cancer shows objective response rates near zero with single-agent or dual checkpoint blockade.

The framework attributes this stark difference to failure-mode patterns. MSI-high CRC has extremely high tumor mutational burden (Mode 1 strongly favorable) and dense T cell infiltration (Mode 3 favorable), so Mode 4 checkpoint blockade alone suffices. MSS CRC has the opposite pattern:

- **Mode 1 (antigen presentation):** Unfavorable. MSS CRC has low tumor mutational burden — far fewer neoantigens than MSI-high tumors. Antigen availability is a primary limitation.
- **Mode 2 (T cell priming):** Unfavorable. Limited dendritic cell engagement; "cold" immunologic phenotype.
- **Mode 3 (physical exclusion):** Unfavorable. MSS CRC is typically poorly infiltrated, with T cells excluded from the tumor core. Stromal and vascular barriers limit T cell access.
- **Mode 4 (T cell exhaustion):** Not the limiting factor. Checkpoint blockade addresses Mode 4, but Mode 4 is not where MSS CRC fails — explaining why checkpoint blockade alone is inactive.
- **Mode 5 (active suppression):** Unfavorable. Treg and MDSC infiltration; immunosuppressive cytokine milieu.
- **Mode 7 (heterogeneity):** Variable; relevant in metastatic disease.

This is the framework's clearest case. MSS CRC fails on Modes 1, 2, 3, 5 simultaneously, with Mode 4 (the mode checkpoint blockade addresses) not being the bottleneck. This perfectly explains why the field's intensive investment in checkpoint blockade combinations has failed in MSS CRC: it has been optimizing the one mode that wasn't broken.

The components for multi-mode engagement already exist within CRC standard of care:

The remarkable feature of MSS CRC is that standard first-line therapy already engages several of the failure modes — they have simply never been combined with the missing modes:

- **FOLFOX (oxaliplatin-based chemotherapy)** induces immunogenic cell death, engaging Modes 1 and 7 — oxaliplatin is one of the most reliably immunogenic chemotherapies, exposing neoantigens and danger signals.
- **Bevacizumab (anti-VEGF)** is standard in metastatic CRC and engages Mode 3 via vascular normalization, improving T cell access — the same mechanism demonstrated in IMbrave150 (Finn et al., *NEJM* 2020) for hepatocellular carcinoma.

So the FOLFOX/bevacizumab standard already engages Modes 1, 3, 7 partially. The framework predicts that adding the missing modes — Mode 2 (T cell priming), Mode 4 (checkpoint blockade), Mode 6 (persistence) — to this existing backbone could convert MSS CRC from immunotherapy-refractory to immunotherapy-responsive.

This has never been systematically tested. Checkpoint blockade has been added to FOLFOX/bevacizumab (engaging Mode 4) with marginal results — predictably, because Mode 4 was not the bottleneck. But adding Mode 2 + Mode 4 + Mode 6 together to the existing Mode 1/3/7 backbone produces combinatorial-complete coverage that has not been attempted.

4.2 Proposed intervention: Multi-mode protocol on the FOLFOX/bevacizumab backbone

Trial name (proposed): BREAKTHROUGH-3 (Broadened Response by Engaging All seven modes in microsatellite-stable colorectal cancer — referred to as **BREAKTHROUGH-3** or the **multi-mode MSS CRC protocol**).

Intervention arm:

First-line systemic therapy:

1. **FOLFOX** (folinic acid, fluorouracil, oxaliplatin) — engages Modes 1, 7 via immunogenic cell death; standard backbone
2. **Bevacizumab** (anti-VEGF) — engages Mode 3 via vascular normalization; standard backbone
3. **NEW — Checkpoint blockade for Mode 4:** Anti-PD-1 (pembrolizumab or nivolumab) — engages Mode 4. Previously tested with marginal results as a single addition; here combined with the missing modes.
4. **NEW — Intratumoral TLR agonist for Mode 2:** Intratumoral poly-ICLC or equivalent TLR agonist, injected into accessible metastases — engages Modes

2 and 3 via in situ dendritic cell activation and inflammatory disruption of the excluded microenvironment. The Hammerich 2019 (*Nature Medicine*) in situ vaccination data demonstrate that intratumoral TLR agonist + checkpoint blockade can generate systemic (abscopal) responses in otherwise immunotherapy-resistant tumors.

5. **NEW — IL-15 superagonist for Mode 6:** N-803 (ANKTIVA) — engages Mode 6 via memory T cell support and sustained engagement. Hurton et al. (*PNAS* 2016) demonstrated IL-15's centrality to T cell persistence.

Mechanistic logic: The intratumoral TLR agonist (Mode 2) generates dendritic cell activation and in situ antigen presentation using the neoantigens released by FOLFOX-induced immunogenic cell death (Mode 1). Bevacizumab-mediated vascular normalization (Mode 3) permits the resulting T cells to access tumors. Checkpoint blockade (Mode 4) prevents exhaustion of these newly primed T cells. IL-15 superagonist (Mode 6) sustains them as memory cells. This is combinatorial-complete coverage applied to the existing CRC standard of care.

Sequencing principle (per Section 1.6): FOLFOX is one of the most reliably immunogenic chemotherapy regimens (oxaliplatin is a prototypical inducer of immunogenic cell death), which makes it well-suited to serve as an immune *trigger* rather than an immune suppressant — provided the immune-priming components are active concurrently. Accordingly, the intratumoral TLR agonist (the modern-Coley danger signal) and checkpoint blockade begin with the first cycle, so that dendritic-cell activation and T-cell priming are underway as oxaliplatin-induced immunogenic cell death releases antigens, not afterward. The IL-15 superagonist (Mode 6) is sustained throughout to protect the primed population across successive chemotherapy cycles. FOLFOX is delivered at standard or, where clinically appropriate, response-guided dosing rather than escalated to maximal myelosuppression, consistent with the principle that the regimen should trigger and not flatten the immune response. This immune-concurrent sequencing distinguishes the protocol from prior MSS CRC attempts that added checkpoint blockade to chemotherapy without an intratumoral priming signal and without attention to preserving immune competence.

Comparator arm: Standard FOLFOX + bevacizumab (current first-line standard for MSS metastatic CRC).

Optional third arm: FOLFOX + bevacizumab + checkpoint blockade alone (testing whether the previously-marginal Mode 4 addition contributes within the full combination, and isolating the contribution of the Mode 2 + Mode 6 additions).

4.3 Mode-by-mode coverage analysis (full intervention arm)

Mode	Engagement	Component(s)	Coverage score
1: Antigen presentation	Moderate-strong	FOLFOX (immunogenic cell death) + intratumoral inflammation	0.70
2: T cell priming	Moderate-strong	Intratumoral TLR agonist (poly-ICLC)	0.75

Mode	Engagement	Component(s)	Coverage score
3: Physical exclusion	Moderate	Bevacizumab (vascular normalization) + intratumoral inflammation	0.70
4: T cell exhaustion	Strong	Anti-PD-1	0.90
5: Active suppression	Low-moderate	FOLFOX MDSC effects + inflammatory disruption	0.45
6: Persistence	Strong	N-803 IL-15 superagonist	0.85
7: Tumor heterogeneity	Moderate	FOLFOX broad cytotoxicity + intratumoral polyclonal priming	0.65

The full intervention arm achieves combinatorial coverage across all seven modes, with Mode 5 (active suppression) the weakest point at 0.45. This identifies where a future iteration might add a CD73/A2A inhibitor or Treg-depleting agent. The framework predicts even this six-strong-mode coverage (Mode 5 partial) will produce qualitative improvement over the FOLFOX/bevacizumab standard's coverage (Modes 1, 3, 7 partial; Modes 2, 4, 5, 6 essentially absent).

4.4 Endpoints

Primary endpoint: Progression-free survival (PFS), comparing full intervention arm to FOLFOX/bevacizumab standard.

Standard first-line FOLFOX/bevacizumab achieves median PFS of approximately 10-11 months in metastatic MSS CRC. The framework predicts the multi-mode protocol will meaningfully extend PFS and, critically, produce a tail of durable responders — the hallmark of effective immunotherapy that is currently entirely absent in MSS CRC.

Key secondary endpoints: - Overall survival (OS) - Objective response rate (ORR) — currently near zero with checkpoint blockade in MSS CRC; the framework predicts measurable ORR with combinatorial-complete coverage - Duration of response (testing for the durable-responder tail) - Response in injected vs non-injected lesions (abscopal effect, testing systemic immunity generation) - Landmark PFS and OS at 1, 2, 3 years (testing for durable benefit tail) - Safety and toxicity

Exploratory endpoints (correlative science): - Tumor immune infiltration dynamics (baseline vs on-treatment biopsy) — tests whether the cold MSS microenvironment converts to inflamed - T cell infiltration in injected vs non-injected lesions — tests systemic immunity generation - Neoantigen-specific T cell responses (despite low TMB)

— tests whether intratumoral priming generates responses against the limited MSS antigen repertoire - ctDNA dynamics - Tumor microenvironment single-cell evolution

4.5 Statistical design

Trial design: Randomized phase II, two arms (or three with the checkpoint-only arm), with phase III expansion conditional on results.

Sample size estimation: Assuming median PFS of 10.5 months with FOLFOX/bevacizumab standard and framework-predicted improvement to 15 months with the multi-mode protocol (hazard ratio approximately 0.65), with the more important endpoint being the durable-responder tail: - Per arm: n = 100 - Total phase II (two-arm): n = 200, or n = 300 for three-arm design

The trial is powered both for the PFS difference and, importantly, for detection of a durable-responder subpopulation (e.g., ≥15% of intervention-arm patients with PFS >24 months versus ~0% in control), which would be the qualitative signal that MSS CRC has been converted to immunotherapy-responsive.

4.6 Biomarker strategy

Pre-specified biomarker hypotheses:

1. **The intervention arm will produce a durable-responder subpopulation absent in the control arm.** This is the central qualitative test. MSS CRC currently produces essentially no durable immunotherapy responders. If the multi-mode protocol produces even a 15-20% durable-responder tail, the framework's prediction — that combinatorial-complete coverage converts refractory status — is validated.
2. **Abscopal responses (regression of non-injected lesions) will correlate with intratumoral TLR agonist-induced systemic immunity.** Tests the Mode 2 contribution and systemic immunity generation.
3. **Conversion of cold to inflamed tumor microenvironment (increased CD8 infiltration on-treatment biopsy) will correlate with response.** Tests whether the combinatorial protocol overcomes the Mode 3 exclusion that defines MSS CRC.
4. **Durable responders will show sustained memory T cell populations (IL-15-supported), distinguishing them from transient responders.** Tests the Mode 6 contribution to durability.

4.7 Safety considerations

Anticipated toxicity profile:

All components have established safety profiles: - **FOLFOX + bevacizumab:** Standard, well-characterized (cytopenias, neuropathy, GI toxicity, hypertension, thrombosis). - **Anti-PD-1:** Standard immune-related adverse events. - **Intratumoral poly-ICLC:** Local injection effects, transient fever; requires accessible lesions and inter-

ventional radiology support for deep lesions. - **N-803:** Established mild-moderate constitutional profile.

Combination concerns: - Bevacizumab + intratumoral injection requires attention to bleeding risk at injection sites. - Overlapping immune-related toxicity from checkpoint blockade + TLR agonist + IL-15 superagonist requires monitoring. - Intratumoral injection logistics (interventional radiology for liver/deep lesions) add operational complexity.

Pre-specified safety rules, DSMB oversight, and interim analyses as in the other trials.

4.8 Feasibility analysis

Component availability:

Component	Status	Manufacturer
FOLFOX	FDA-approved, generic	Multiple
Bevacizumab (Avastin)	FDA-approved for mCRC	Genentech/Roche
Anti-PD-1 (pembrolizumab/nivolumab)	FDA-approved	Merck / BMS
Poly-ICLC (Hiltonol)	Investigational, multiple trials	Oncovir
N-803 (ANKTIVA)	FDA-approved for NMIBC	ImmunityBio

Multi-sponsor coordination: Requires checkpoint inhibitor sponsor (Merck or BMS) + Genentech (bevacizumab) + Oncovir (poly-ICLC) + ImmunityBio (ANKTIVA). The components are widely available; bevacizumab and FOLFOX are generic/standard.

Operational considerations: The intratumoral injection component requires accessible lesions and interventional radiology infrastructure — a feasibility constraint that limits eligible patients to those with injectable metastases but is manageable at major cancer centers.

Estimated trial cost: $20-35 million for phase II.

Estimated trial duration: 8-10 years total.

4.9 Why this trial should be run now — the largest opportunity

MSS colorectal cancer is the single largest immunotherapy-refractory population in oncology. Over 100,000 patients per year in the United States, and the majority of the global colorectal cancer burden, derive essentially zero benefit from the immunotherapy revolution that has transformed melanoma, lung cancer, and other malignancies.

The framework's analysis is unusually clear in this case. MSS CRC fails on Modes 1, 2, 3, 5 simultaneously, with Mode 4 — the mode checkpoint blockade addresses — not being the bottleneck. This explains, with structural precision, why a decade of checkpoint blockade combination trials has failed in MSS CRC: the field has been intensively optimizing the one mode that was not the limiting factor.

The framework predicts that combinatorial-complete coverage — adding Mode 2 (intratumoral priming), Mode 4 (checkpoint), and Mode 6 (IL-15 persistence) to the existing FOLFOX/bevacizumab backbone that already provides Mode 1, 3, 7 coverage — could convert MSS CRC from completely refractory to measurably responsive, producing the durable-responder tail that is the signature of effective immunotherapy and is currently entirely absent.

The components exist. Most are FDA-approved. The backbone (FOLFOX/bevacizumab) is the existing standard of care. The additions are individually established agents. The trial requires combining them in the combinatorial-complete pattern the framework specifies.

If the framework is correct, BREAKTHROUGH-3 would represent the largest single expansion of effective immunotherapy to a refractory population in the field's history — bringing durable immune-mediated responses to a fraction of the largest immunotherapy-refractory cancer population.

This is the highest-impact trial the framework identifies. It should be run.

5. Cross-trial design considerations

The three trials proposed above share structural features that warrant unified discussion. These considerations apply to any combinatorial-complete protocol testing the seven-mode framework, not only to the three specific trials proposed.

5.1 The deconvolution problem

The most significant methodological objection to combinatorial-complete trials is that they cannot determine which component is responsible for benefit. A protocol combining five or six agents that outperforms standard of care does not reveal whether all components are necessary, whether some are inert, or whether benefit derives from one or two key additions.

This objection is real but, in the framework's view, secondary at the proof-of-principle stage. The first question is whether combinatorial-complete coverage produces qualitatively different outcomes than single-mode or partial-combination approaches. If it does not, deconvolution is moot. If it does, deconvolution becomes the subject of subsequent factorial trials.

The framework's prediction is specifically about combinatorial completeness, not about individual components. The hypothesis is that engaging all seven modes simultaneously crosses a threshold that engaging subsets does not — analogous to combination antiretroviral therapy in HIV, where the combination achieves durable viral suppression that no individual agent or pair achieves, and where the historical sequence was establishing combination efficacy first and optimizing individual components second.

The three-arm designs proposed for Trials 2 and 3 provide partial deconvolution by comparing the combinatorial-complete arm against both standard of care and

the established partial-combination backbone. This isolates the contribution of the framework-guided additions without requiring a full factorial design.

Full factorial deconvolution — testing every subcombination — would require sample sizes an order of magnitude larger and is appropriately deferred to confirmatory trials after proof-of-principle.

5.2 Toxicity and the additive-adverse-event concern

Combining five or six immunologically active agents raises legitimate concern about cumulative toxicity, particularly overlapping immune-related adverse events. Checkpoint blockade, IL-15 superagonists, TLR agonists, and personalized vaccines each carry immune-activation toxicity; in combination, the risk of severe immune-related adverse events, cytokine release, and autoimmune phenomena could be more than additive.

Several considerations mitigate this concern:

First, the component agents have established individual safety profiles, and several combinations have already been tested without prohibitive toxicity. Checkpoint blockade + vaccine (KEYNOTE-942, autogene cevumeran), checkpoint blockade + IL-15 superagonist (in development), and intratumoral TLR agonist + checkpoint blockade (Hammerich 2019) have all been tested with manageable toxicity.

Second, several components engage immunity through non-overlapping mechanisms with potentially non-overlapping toxicity. Vascular normalization (bevacizumab), adenosine inhibition (CD73 inhibitors), and intratumoral local therapy have toxicity profiles largely distinct from systemic checkpoint blockade.

Third, the trials incorporate pre-specified safety stopping rules, frequent DSMB review, and staged enrollment with interim safety analyses, allowing early detection of unacceptable cumulative toxicity.

The framework does not assume that combinatorial-complete protocols will be non-toxic. It predicts that the therapeutic benefit of combinatorial completeness will justify manageable increases in toxicity, as has been the case for combination checkpoint blockade (ipilimumab + nivolumab carries substantially higher toxicity than monotherapy but is justified by superior efficacy in appropriate populations).

5.3 Sequencing and timing

The order and timing of component administration may matter substantially — in some cases decisively. The foundational sequencing and chemotherapy-dosing principle is stated in Section 1.6; this section elaborates the mode-specific guidance that follows from it.

The cardinal rule: establish immunity before, or concurrent with, cytoreduction — never destroy a primed response with mistimed myelosuppression. This is the single most important sequencing consideration and the one most often violated in conventional combination trials, which typically deliver full-dose chemotherapy first and add immunotherapy as an afterthought. Because activated, clonally

expanding anti-tumor T cells are rapidly dividing and therefore vulnerable to cytotoxic chemotherapy, high-dose myelosuppressive chemotherapy delivered during the priming/expansion window can abolish the very response the protocol is designed to build.

Mode 1 and 7 engagement (immunogenic cell death via chemotherapy or radiation) should coincide with active Mode 2 engagement (DC activation), so that antigens released by dying tumor cells are captured by already-activated dendritic cells. The Hammerich in situ vaccination protocol explicitly sequences Flt3L (DC recruitment) → radiotherapy (antigen release) → TLR agonist (DC activation) for this reason. Immunogenic-dose chemotherapy is the trigger; the priming machinery must be switched on when the trigger is pulled.

Mode 4 engagement (checkpoint blockade) should coincide with or follow the onset of T cell priming (Modes 1, 2), so that newly primed T cells are protected from exhaustion. Checkpoint blockade administered before any T cell priming has nothing to act upon.

Mode 6 engagement (IL-15 persistence support) should span and outlast the chemotherapy phase, both to sustain established responses as memory cells and — critically — to protect the primed T cell pool through any necessary myelosuppressive chemotherapy window and into the maintenance/adjuvant phase.

Mode 3 engagement (stromal/vascular modification) should precede or coincide with T cell-generating interventions, so that the T cells generated can access the tumor.

Chemotherapy dose, not only timing, matters. Where a choice exists, immunogenic and less-myelosuppressive agents (oxaliplatin, platinum/taxane combinations) are preferred as immune triggers over maximally myelosuppressive regimens; and assay-guided or response-guided dosing — tailoring chemotherapy to the minimum effective dose for a given tumor — is preferable to fixed maximal dosing because it preserves the immune competence on which the combinatorial strategy depends. The proposed trials therefore treat conventional fixed-dose chemotherapy backbones as starting points to be modified by these principles, not as fixed constraints, and in Trial 1 the most myelosuppressive (anthracycline) phase is explicitly repositioned, protected, or conditionally de-escalated for this reason.

These sequencing principles are incorporated into the proposed trial schemata but also represent testable hypotheses. Optimal sequencing is an empirical question that proof-of-principle trials can begin to address through correlative analysis and that subsequent trials can optimize. The autogene cevumeran result in PDAC (Trial 2), achieved with a deliberately immune-first sequence, provides early clinical support that the principle is real and not merely theoretical.

5.4 Biomarker-driven patient selection

The three trials all incorporate extensive correlative biomarker programs. Beyond their scientific value in testing the framework's mechanisms, these programs serve a translational purpose: identifying which patients benefit most from combinatorial-complete protocols.

The framework predicts that the benefit of engaging a particular mode depends on whether that mode is a failure mode in a given patient's tumor. A patient whose tumor has intact antigen presentation (Mode 1 favorable) derives less benefit from Mode 1 engagement than a patient with antigen presentation defects. This implies that combinatorial-complete protocols might eventually be personalized — each patient receiving engagement of precisely the modes that are failing in their specific tumor.

The proposed trials are not yet personalized in this way; they apply uniform combinatorial-complete protocols to defined populations. But the biomarker data they generate would enable subsequent personalized-combination trials, in which each patient's failure-mode profile (measured by the correlative assays) determines their specific combination. This is the framework's long-term translational vision: failure-mode profiling followed by precision combination immunotherapy.

5.5 Statistical philosophy: detecting qualitative conversion

The conventional statistical framework for oncology trials emphasizes incremental improvements in median survival endpoints. The seven-mode framework predicts a different kind of effect: qualitative conversion of refractory populations into responsive ones, manifesting as the emergence of durable-responder tails rather than uniform shifts in median survival.

This has statistical design implications. The trials are powered not only for median PFS/EFS/RFS differences but specifically for detection of durable-responder subpopulations — the long-term survival plateau that is the signature of effective immunotherapy. In MSS CRC particularly, the qualitative signal (emergence of any durable responders in a population that currently has essentially none) may be more important than the median PFS difference.

Landmark analyses (PFS and OS at 1, 2, 3 years), restricted mean survival time, and explicit modeling of the survival-curve tail are incorporated alongside conventional median-based endpoints. The framework's prediction is fundamentally about the tail of the survival distribution, and the analysis plans reflect this.

5.6 Regulatory and developmental pathway

The proposed trials are phase II proof-of-principle studies. Their purpose is to establish whether combinatorial-complete coverage produces the qualitative benefit the framework predicts. Positive results would justify phase III confirmatory trials and, given the magnitude of unmet need in the target populations, might support accelerated regulatory pathways.

The multi-component, multi-sponsor nature of these trials presents the principal developmental obstacle. No single pharmaceutical sponsor owns all components of any proposed protocol. Academic consortia, cooperative groups, and government-sponsored trial networks (NCI's National Clinical Trials Network) are better positioned than individual companies to run combinatorial-complete trials spanning multiple sponsors' products. Philanthropic and government funding may be necessary to complement industry contributions of investigational agents.

This developmental structure — academic/cooperative-group-led, multi-sponsor-supplied, philanthropically/governmentally co-funded — is unusual but not unprecedented. It is, in the framework's view, the necessary developmental model for testing combinatorial-complete protocols, because the combinatorial logic crosses the proprietary boundaries that individual-sponsor trials respect.

6. Discussion

6.1 What these trials would establish

The three trials proposed in this paper share a single underlying purpose: to test the seven-mode framework's central prediction that combinatorial-complete coverage produces qualitatively different clinical outcomes than single-mode or partial-combination approaches.

If the trials succeed — if combinatorial-complete protocols produce the predicted qualitative improvements (higher pCR in BRCA1/2-mutant TNBC, extended vaccine-responder fraction in PDAC, durable-responder tail in MSS CRC) — they would establish combinatorial completeness as an organizing principle for immunotherapy development. The implication would extend far beyond the three tested cancers: the framework would provide a systematic method for designing combination protocols in any immunotherapy-refractory cancer, by profiling the failure modes and engaging those that are operative.

If the trials fail — if combinatorial-complete coverage produces no benefit beyond standard of care or partial combinations — the framework's central prediction would be refuted, and the field would have learned that the failure-mode model, however appealing structurally, does not translate into clinical benefit through the combinatorial strategy proposed. This is a real and acceptable possibility. The framework is falsifiable, and these trials are the falsification test.

Either outcome advances the field. The current situation — in which combinatorial-complete protocols are predicted by a structural framework to produce qualitative benefit but have never been systematically tested — is the unsatisfactory state these trials would resolve.

6.2 Why these trials have not been run

The components for combinatorial-complete protocols have existed, in growing completeness, for several years. The biological rationale for cross-mode combination has been articulated in various forms by multiple investigators. Yet the specific combinatorial-complete trials proposed here, or close equivalents, have not been run. Why?

Several structural reasons, articulated in Paper 3 and reinforced here:

Proprietary fragmentation. No single sponsor owns the full component set for any combinatorial-complete protocol. The combinatorial logic crosses corporate boundaries that individual-sponsor development respects. Multi-sponsor trials are difficult

to organize, and the incentives for any single sponsor to lead a trial whose benefit might be attributed to a competitor's component are weak.

Disciplinary fragmentation. The expertise required to design combinatorial-complete protocols spans checkpoint biology, vaccine immunology, stromal biology, cytokine engineering, oncolytic virology, and adoptive cell therapy. Few individual investigators command this full range. The integration the framework requires is rare.

The single-mechanism trial paradigm. Contemporary trial design strongly favors testing one new agent or mechanism at a time, against a standard backbone, to isolate that agent's contribution. This paradigm — methodologically rigorous for individual agents — structurally precludes the combinatorial-complete approach, which deliberately combines many mechanisms at once.

Toxicity caution. Reasonable concern about cumulative toxicity discourages combining many active agents, particularly when the benefit is hypothesized rather than established.

Statistical conservatism. The large sample sizes required for fully deconvolved factorial designs discourage combinatorial trials; the proof-of-principle designs proposed here (combinatorial-complete vs standard, without full deconvolution) are less familiar and may face methodological skepticism.

These obstacles are structural, not scientific. The science supports the trials. The structures of pharmaceutical development, academic specialization, and trial-design convention have prevented them.

6.3 The role of frameworks in overcoming structural obstacles

A framework's value, beyond its descriptive utility, is its capacity to overcome structural obstacles by making the case for trials that the existing structures would not generate.

The seven-mode framework makes the case for combinatorial-complete trials in three ways. It provides the rationale — a structural argument for why combinatorial completeness should matter. It provides the design method — the mode-by-mode coverage analysis that specifies which components engage which modes and identifies coverage gaps. And it provides the falsification criterion — a clear prediction (qualitative conversion of refractory populations) that the trials can confirm or refute.

This paper translates the framework into three specific, feasible, high-impact trial designs. The translation is deliberately concrete: named components, defined populations, specified endpoints, statistical designs, feasibility analyses. The intent is to lower the barrier to implementation by doing the design work that the structural obstacles have prevented.

6.4 Limitations

This paper has significant limitations that must be stated plainly.

These are proposed trials, not conducted trials. No data are presented. The framework's predictions are hypotheses. The trials might fail. Every claim about predicted benefit is a prediction, not a result.

The author is an independent researcher without institutional affiliation, clinical trial experience, or the infrastructure to conduct these trials. The trial designs are necessarily provisional and would require substantial refinement by experienced clinical trialists, biostatisticians, and regulatory experts before implementation. Sample size estimates are approximate. Dosing and scheduling details require expert refinement. Safety considerations require formal review.

The component selections reflect the framework's logic but are not the only possible choices. Other agents could engage the same modes. The specific components proposed (particular checkpoint inhibitors, particular IL-15 superagonists, particular stromal-modifying agents) are illustrative of the combinatorial-complete approach, not uniquely determined by it.

The framework itself may be incorrect or incomplete. Paper 3 develops the framework's rationale, but the framework is a structural hypothesis, not an established fact. These trials test the framework; they do not assume its correctness.

The deconvolution limitation is real. Even successful trials would not fully establish which components are necessary, requiring subsequent factorial studies.

Multi-sponsor coordination, manufacturing logistics, and funding represent substantial practical obstacles that this paper acknowledges but does not solve.

These limitations do not, in the author's view, diminish the core argument: that combinatorial-complete protocols are biologically rational, operationally feasible with existing components, predicted by a structural framework to produce qualitative benefit, and have not been systematically tested. The limitations bear on how the trials should be designed and conducted, not on whether they should be.

6.5 Conclusion

The seven-mode framework predicts that combinatorial-complete immunotherapy protocols — engaging all seven failure modes of immune-mediated tumor control simultaneously — will convert immunotherapy-refractory cancers into immunotherapy-responsive ones. This paper has translated that prediction into three specific, feasible trial designs in three large unmet-need populations: BRCA1/2-mutant triple-negative breast cancer, resectable pancreatic ductal adenocarcinoma, and microsatellite-stable colorectal cancer.

Each trial builds on an existing standard or emerging positive signal, adds the framework-identified missing modes using FDA-approved or late-stage components, and tests whether combinatorial completeness produces the predicted qualitative benefit. Each is biologically rational, operationally feasible, and unprecedented in systematic form.

The components exist. The rationale is established. The populations are defined and large. The endpoints are meaningful and achievable. The obstacles are structural

— proprietary fragmentation, disciplinary specialization, trial-design convention — rather than scientific.

These trials should be run. The framework predicts they will work. If they do, they would represent among the largest expansions of effective immunotherapy to refractory populations in the field's history. If they do not, they would refute a framework that currently stands untested. Either outcome is worth the trials.

The empirical observation that motivated the framework — that combinatorial multimode treatment can produce durable benefit in cancers that single-mode treatment cannot reach — was made in individual cases, including the author's family experience with BRCA1-mutant triple-negative breast cancer, before the framework existed to describe it. The structural completion of that observation is the framework. The clinical test of that observation is these trials. They remain to be run.

The author declares no financial conflicts of interest. No funding was received for this work. *The author is an independent researcher without institutional affiliation. Correspondence: eric@miacreativeagency.com. ORCID: 0009-0003-6805-1381.*

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Note on citation reuse

Paper 4 draws substantially on the verified citation base established for Paper 3 of this series. The two papers should be read together: Paper 3 establishes the framework and its supporting literature; Paper 4 translates the framework into trial designs. The citation overlap reflects this deliberate continuity. New citations introduced specifically for Paper 4 (the KEYNOTE-522 primary and EFS analyses, the oleclumab first-in-human study with full bibliographic details, and the epidemiologic prevalence figures for BRCA1/2-mutant TNBC and MSS CRC) were verified independently for this paper.